Supporting Information

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SI Methods

Mutant Hsp104 Strains. Mutant Hsp104 strains were constructed as follows: W303 *hsp104*-Δ [39] was crossed to YJW616 [*psi*$^-$] [*rnq*$^-$], formed by treatment with GdnHCl, and sporulated to obtain a [*psi*$^-$] [*rnq*$^-$] *hsp104*-Δ *ade1–14*, *ADE2* haploid. This strain was transformed by plasmids bearing either *HSP104* or *hsp104-T106M* under the control of the *HSP104* promoter. Resulting transformants were crossed with *sis1*-Δ::*LEU2* [*TETrSIS1*] [*PSI*$^+$] [*rnq*$^-$] strain and dissected to obtain a [*PSI*$^+$] [*rnq*$^-$] *sis1*-Δ *hsp104*-Δ [*TETrSIS1*] [*hsp104-T160M*] strain and the analogous strain with the WT *HSP104* gene.

Kinetic Models for Prion Curing. Kinetic equations used to fit prion curing data were derived from the expression $p_0 = e^{-m}$, where p_0 is the fraction of cells in any generation that are [*prion*$^-$], and m is the average number of seeds per cell in the population (13). In a curing experiment, the value of m varies with generation number (g) as $m = N_o/f^g$, where N_o is the initial value of m at $g = 0$, and f is a variable that ranges from $1 \leq f \leq 2$, here defined as the "fragmentation factor" (discussed later). The combination of these two expressions yields an equation that describes p_0 for all non-negative values of g:

$$P_0 = e^{\frac{-N_o}{f^g}}$$

Experimental values of N_o and f for [*PSI*$^+$] curing experiments were determined by linear least-squares regression analysis. Data were fit to a linear form of the Eq. 1: log (ln (p_0)) = (−log (f)) × (g) + log (N_o) Only data corresponding to 2–98% [*PSI*$^+$] ($0.98 \geq p_0 \geq 0.02$) were used in data fitting [13]. A value of N_o was determined by fitting data from GdnHCl curing experiments assuming $f = 2$ [13, 22, 26]. Subsequently, experimentally derived values of N_o were held constant in fits to determine the value of f in *SIS1*-repression curing experiments. Regression lines for all fits had R^2 values ≥ 0.9.

Fragmentation Efficiency. Previous analyses of [*PSI*$^+$] or [*URE3*] curing by GdnHCl treatment assumed a fragmentation efficiency of 0% based on the complete inhibition of Hsp104 where $f = 2$ and the average seed number in the population (m) is halved each generation (13, 22, 26). Alternatively, the assumption that $f = 1$ results in a steady-state condition in which seed number remains constant; $m = N_o$ for all g. We define this condition to be 100% fragmentation, meaning that, on average, 100% of all seeds are fragmented exactly once per cell generation. Because it is undesirable to suggest that every seed is fragmented once or not at all, we suggest that fragmentation efficiency can also be interpreted as the sum total number of fragmentation events that produce a new seed, divided by the total initial number of seeds in any generation. Thus, at 100% fragmentation, a cell containing 100 seeds would undergo 100 fragmentation events producing 100 additional seeds; following cell division, both mother and daughter cells retain 100 seeds. Fragmentation efficiency, expressed as a percentage, can be easily calculated for any value of f from the equation: *fragmentation efficiency* = ([2/f] − 1) × (100%)

1. Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. *Biophotonics Int* 11:36–42.

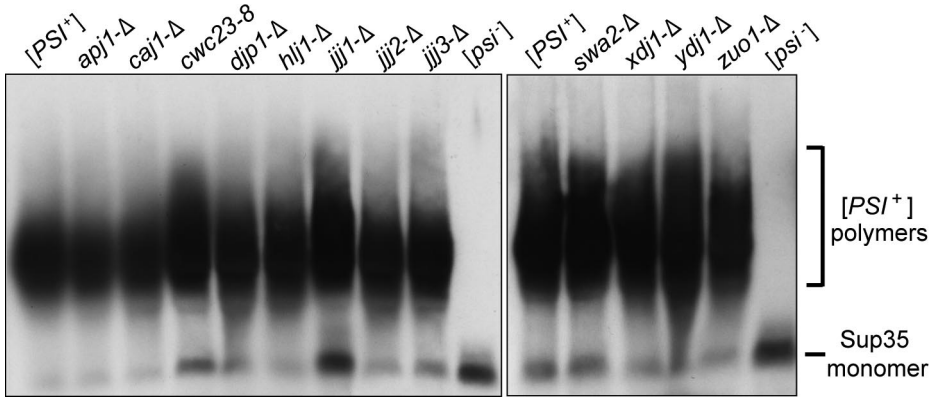

Fig. S1. No J-protein other than Sis1 is required for maintenance of [*PSI*$^{+}$]. [*PSI*$^{+}$] cells lacking individual J-proteins were passaged for 3 weeks on rich media before analysis. Cell lysates were prepared, resolved by SDD-AGE, and subjected to immunoblotting using Sup35-specific antibodies. Control WT [*PSI*$^{+}$] and [*psi*$^{-}$] cells were included for comparison.

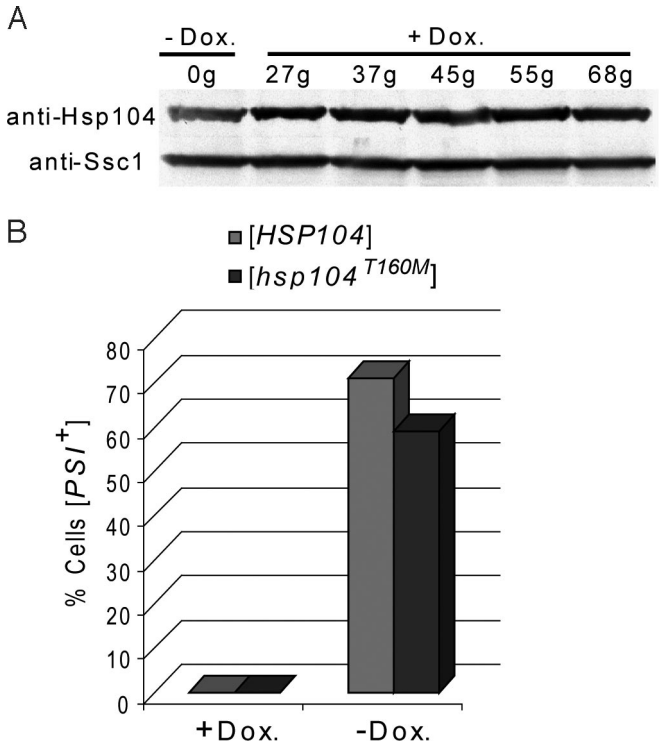

Fig. S2. Alteration of Hsp104 expression upon Sis1 depletion is not a causative factor in [*PSI*$^{+}$] curing. (*A*) Lysates from *sis1*-Δ [*TETrSIS1*] cells grown for the indicated number of generations in the presence of doxycycline were separated by SDS/PAGE and visualized by immunoblotting with Hsp104-specific antibodies and Ssc1-specific antibodies (loading control). Hsp104 levels (quantified with ImageJ) (1) were elevated less than twofold upon Sis1 depletion. (*B*) *sis1*-Δ [*TETrSIS1*] cells expressing WT Hsp104 or Hsp104^{T160M} were grown on doxycycline-containing media to repress *SIS1* expression. To prevent curing by spontaneous loss of the non-essential plasmid, cells were first plated onto solid medium selective for the plasmids (−HIS) and grown overnight before replica-plating colonies onto media lacking adenine (−ADE). The fraction of colonies which grew in the absence of adenine, which reflects the fraction of [*PSI*$^{+}$] cells in the population, is shown at 60 generations.

Table S1. Cytoduction and lysate transformation assays of cells before (0 generations) or after (118 generations) Sis1 depletion

	Fraction [*PSI*$^+$]	
	No Doxycycline (0 generations)	Doxycycline (118 generations)
Cytoduction	12/12 (100%)	0/12 (0%)
Lysate transformation	47/55 (85%)	0/110 (0%)